Patent
Attorney's Docket No. 031786-046

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING CONTINUATION/DIVISIONAL APPLICATION UNDER 37 C.F.R. § 1.53(b)

Box PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a request for filing a [ ] continuation [x] divisional application under 37 C.F.R. § 1.53(b) of pending Application No. 09/091,521 filed on June 19, 1998, for AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME, by the following named inventor(s):

(a) Full Name Stephen M. Boyle
(b) Full Name Silvio Cravero
(c) Full Name Lynette Corbeil
(d) Full Name Gerhardt Schurig
(e) Full Name Nammalwar Srirnaganathan
(f) Full Name Ramesh Vemulapalli

[x] The entire disclosure of the prior application from which a copy of the oath or declaration is supplied herewith is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

[ ] This application is being filed by less than all the inventors named in the prior application. In accordance with 37 C.F.R. 1.63(d)(2), the Commissioner is requested to delete the name(s) of the following person or persons who are not inventors of the invention being claimed in this application.

(a) Full Name _____
(b) Full Name _____
(c) Full Name _____

[ ] This application is being filed by more than all the inventors named in the prior application. In accordance with 37 C.F.R. 1.63(d)(2), the Commissioner is requested to add the name(s) of the following person or persons who are inventors of the invention being claimed in this application.

21839

(a) Full Name _____

(b) Full Name _____

(c) Full Name _____

1. [x] Enclosed is a copy of the prior Application No. 09/091,521 as originally filed on June 19, 1998, including copies of the specification, claims, drawings and the executed oath or declaration as filed.

2. [ ] Enclosed is a revised prior application and a copy of the prior executed oath or declaration as filed. No new matter has been added to the revised application.

3. [x] Small entity status is hereby claimed.

4. [x] The filing fee is calculated below [x] and in accordance with the enclosed preliminary amendment:

CLAIMS					
	NO. OF CLAIMS		EXTRA CLAIMS	RATE	FEE
Basic Application Fee					$710.00 (101)
Total Claims	10	MINUS 20 =	0	× $18.00 (103) =	0
Independent Claims	1	MINUS 3 =	0	× $80.00 (102) =	0
If multiple dependent claims are presented, add $270.00 (104)					
Total Application Fee					
If small entity status is claimed, subtract 50% of Total Application Fee					355.00
Add Assignment Recording Fee of if Assignment document is enclosed					
TOTAL APPLICATION FEE DUE					**355.00**

5. [x] Charge $ 355.00 to Deposit Account No. 02-4800 for the fee due.

6. [ ] A check in the amount of $ _____ is enclosed for the fee due.

7. [X] The Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in duplicate.

8. [ ] Cancel in this application original claims _ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

9. [x] Amend the specification by inserting before the first line the sentence: --This application is a [ ] continuation, [x] divisional, of Application No. 09/091,521, filed June 19, 1998, which is a national stage application of PCT/US97/23032 filed December 5, 1997.--

10. [ ] New drawings are enclosed.

11. [ ] Priority of Application No. _ filed on _ in _ (country) is claimed under 35 U.S.C. § 119.

[ ] The certified copy of the priority application
[ ] is enclosed
[ ] was filed on _ in prior Application No. _, filed on _, and acknowledged by the Examiner on _ in Paper No. _
[ ] has not yet been filed.

12. [x] A preliminary amendment is enclosed.

13. [x] An Information Disclosure Statement is enclosed.

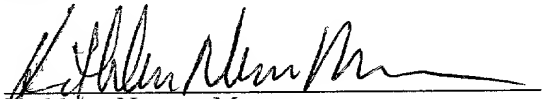
14. [ ] A General Authorization for Payment of Fees and Petitions for Extensions of Time is enclosed.

15. [x] Also enclosed Copies of Verified Small Entity Declarations filed in the parent application, 09/091,521.

16. [x] The power of attorney in the prior application is to Brian P. O'Shaughnessy, Reg. No. 32,747 and Kathleen Neuner Manne, Reg. No. 40,101.
a. [x] The power appears in the original papers in the prior application.
b. [ ] Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
c. [ ] Recognize as Associate Attorney _.
d. [ ] Address all future communications to: (May only be completed by applicant, or attorney or agent of record.)

Brian P. O'Shaughnessy
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404

Date: October 20, 2000

By: 
Kathleen Neuner Manne
Registration No. 40,101

ADDRESS OF SIGNATOR:

BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
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(703) 836-6620

[ ] inventor(s)
[ ] assignee of complete interest
[x] attorney or agent of record
[ ] filed under 37 C.F.R. § 1.34(a)

Patent
Attorney's Docket No. 031786-019

Applicant or Patentee: Stephen M. Boyle et al.

Application or Patent No.: 09/091,521

Filed or Issued: June 19, 1998

For: AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§ 1.9(f) AND 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:
[x] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN University of California San Diego

ADDRESS OF CONCERN 9500 Gilman Drive, La Jolla, California 92093

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 1.21 for purposes of paying reduced fees under Sections 41(a) and 41(b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average, over the previous fiscal year of the concern, of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME by inventor(s) Stephen M. BOYLE; Silvio CRAVERO; Lynette CORBEIL; Gerhardt SCHURIG; Nammalwar SRIRNAGANATHAN and Ramesh VEMULAPALLI described in

[ ] the specification filed herewith
[x] Application No. 09/091,521, filed June 19, 1998.
[ ] Patent No. _____, issued _____.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern, or organization having rights to the invention is listed below,* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c), or by any concern that would not qualify as either a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27.)

Application No. 09/091,521
Attorney's Docket No. 031786-019

NAME Virginia Tech Intellectual Properties, Inc.

ADDRESS 1900 Kraft Avenue, Suite 107, Blacksburg, VA 24060

[ ] individual [ ] small business concern [x] nonprofit organization

NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earlier of the issue fee and any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b).)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Dr. Alan Paau

TITLE OF PERSON OTHER THAN OWNER Director, Technology Transfer Office - UCSD

ADDRESS OF PERSON SIGNING 9500 Gilman Drive, Technology Transfer Office

Mail Code 0910, La Jolla, California 92093-0910

SIGNATURE Alan Paau DATE 7/13/98

(12/97)

Patent
Attorney's Docket No. 031786-019

Applicant or Patentee: Stephen M. Boyle et al.

Application or Patent No.: 09/091,521

Filed or Issued: June 19, 1998

For: AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§ 1.9(f) AND 1.27(d)) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION Virginia Tech Intellectual Properties, Inc.

ADDRESS OF ORGANIZATION 1900 Kraft Drive, Suite 107, Blacksburg, Virginia 24060 United States of America

TYPE OF ORGANIZATION

[ ] University or other institution of higher education

[ ] Tax exempt under Internal Revenue Service Code (26 U.S.C. §§ 501(a) and 501(c)(3))

[x] Nonprofit scientific or educational under statute of state of The United States of America
(Name of state Virginia)
(Citation of statute _____)

[ ] Would qualify as tax exempt under Internal Revenue Service Code (26 U.S.C. §§ 501(a) and 501(c)(3)) if located in The United States of America

[ ] Would qualify as nonprofit scientific or educational under statute of The United States of America if located in The United States of America
(Name of state _____)
(Citation of statute _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. § 1.9(e) for purposes of paying reduced fees under Sections 41(a) and 41(b) of Title 35, United States Code, with regard to the invention entitled by inventor(s) Stephen M. BOYLE; Silvio CRAVERO; Lynette CORBEIL; Gerhardt SCHURIG; Nammalwar SPIRNAGANATHAN and Ramesh VEMULAPALLI described in

[ ] the specification filed herewith

[x] Application No. 09/091,521, filed June 19, 1998.

[ ] Patent No. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the above-identified nonprofit organization are not exclusive, each individual, concern, or organization having rights to the invention is listed below,* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an individual inventor under 37 C.F.R. § 1.9(c), or by any concern that would not qualify as either a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27.)

FULL NAME University of California San Diego

ADDRESS 9500 Gilman Drive, LaJolla, California 92093

[ ] individual [ ] small business concern [x] nonprofit organization

FULL NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earlier of the issue fee and any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b).)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Theodore Kohn

TITLE IN ORGANIZATION Director, PAtenting and Licensing

ADDRESS OF PERSON SIGNING 1900 Kraft Drive, Suite 107

Blacksburg, VA 24060 USA

SIGNATURE _____ DATE 7/15/98

Patent
Attorney's Docket No. 031786- 046

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
Stephen M. BOYLE et al.	)	Group Art Unit: 1641
Application No.: (Pending) New U.S. Divisional Application of U.S. Application Ser. No. 09/091,521	)	Examiner: UNASSIGNED
Filed: October 20, 2000	)	
For: AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified matter, please amend the claims as follows.

IN THE CLAIMS:

Please cancel Claims 1-23 without prejudice or disclaimer.

Please amend Claims 24 and 27 as follows:

24. (Amended) A method for immunization, prophylaxis or treatment of a vertebrate at risk of or suffering from a pathogenic micro-organism comprising the steps of:

a) extracting deoxyribonucleic acid from the pathogenic micro-organism;

b) identifying at least one gene encoding at least one antigen from the deoxyribonucleic acid, wherein said at least one antigen is capable of stimulating protective immunity against the pathogenic micro-organism;

c) inserting the at least one gene into a multicopy plasmid capable of replicating and expressing in the pathogenic micro-organism;

d) transforming an attenuated or avirulent strain [version] of the otherwise pathogenic micro-organism with the plasmid to form a vaccine; and

e) administering an effective amount of said vaccine to the vertebrate.

27. (Amended) The method of claim 26, wherein the pathogenic micro-organism is *Brucella* selected from the group consisting of *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. neotomae* and *B. canis*.

Please add new Claims 31-34 as follows:

--31. The method of claim 26, wherein the pathogenic micro-organism is *Mycobacterium*.

32. The method of claim 31, wherein the at least one gene is a GroEL gene.

33. The method of claim 26, wherein the pathogenic micro-organism is *Vibrio*.

34. The method of claim 33, wherein the at least one gene is a ctxB gene.--

REMARKS

Examination and consideration of the above-identified matter in view of the above amendments and the following remarks is respectfully requested.

By this amendment, Claims 24-34 are pending. Claims 1-23 are canceled. Claims 24 and 27 are amended for clarity. New Claims 31-34 are added and are supported by the Specification and claims as originally filed.

Applicants respectfully submit that all pending Claims 24-34 are in condition for allowance. Prompt and favorable action in the form of a Notice of Allowance is thus respectfully requested.

Should the Examiner require anything further, s/he is invited to contact the Applicants' undersigned representative at the telephone number below.

Respectfully submitted,
BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Kathleen Neuner Manne
Registration No. 40,101

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: October 20, 2000

AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME

The invention described herein was made under a grant from the United States Department of Agriculture. Therefore, the U.S. government may have certain rights in this invention.

The invention pertains to an over-expressing homologous antigen vaccine, a method of producing the same, and a method of using the vaccine for prophylaxis or treatment of a vertebrate suffering from or at risk from a pathogen. The vaccine is derived from an attenuated or avirulent version of the pathogen, and over-expresses one or more genes from the pathogen, thereby providing immunity greater than that induced by a vaccine of the same pathogen without over-expression of a gene.

Background of the Invention

Vaccines are used to protect against diseases, which are caused by pathogens. These pathogens are microbial organisms, such as bacteria and viruses, which affect animals, including humans. Vaccines are primarily derived from a pathogen by producing and administering either: a) an attenuated or avirulent version of the pathogen; b) the killed pathogen; c) extracted protective antigens or antigen mixes of the pathogen (homologous antigens); or d) a micro-organism expressing one or more protective antigens encoded by

cloned genes originating in a microbial pathogen different from the vaccine strain (heterologous antigens).

Vaccines for both bacteria and viruses are engineered from microorganisms expressing one or more protective antigens, as described by K. Jones and M. Sheppard in Designer Vaccines, CRC Press (1997). Vaccines are intended to produce an immune response in the recipient consisting of at least one of an antibody mediated or T cell mediated immune response, thereby preventing future infection by a pathogen, or fighting a current pathogenic infection. In particular, vaccines against facultative intracellular pathogens, those growing inside the cells of the infected host, need to induce a strong and appropriate cell mediated immune response. In contrast, vaccines against obligate extracellular pathogens need to induce an appropriate antibody mediated immune response. Often, regardless of the pathogen, an appropriate combined antibody and cellular mediated immune response leads to sufficient protection or relief from infection. In order to achieve this protection or relief from infection, vaccines may express one or more homologous antigens, heterologous antigens, or a combination of both.

Vaccines may be administered to vertebrates both to prevent and treat infection by pathogens. Thus, vaccines are frequently administered to prevent the spread of a disease caused by a pathogen. In particular, herd animals, such as cows, goats, sheep and

swine, are often vaccinated to prevent the spread of a disease among members of the herd. Further, because certain diseases may travel between vertebrates, including travel between various animals and between animals and humans, vaccines are used to prevent the spread of disease between various species, usually by administration to the infected animal and other uninfected animals in the immediate vicinity. However, other animals in the area which are less likely to contract the disease may also be vaccinated as a prophylactic measure. For example, an infected cow and its as yet uninfected herd may be vaccinated to treat a disease and prevent its further spread. As a prophylactic measure, other animals which are likely to contract the disease from the infected cow, such as neighboring cows, sheep or humans, may be vaccinated as well.

It has been found that vaccines derived from an attenuated or avirulent version of a pathogen are highly effective in preventing or fighting disease caused by that pathogen. In particular, it is known that such attenuated or avirulent pathogens can be modified to express heterologous antigens (antigens which are derived from a pathogen of a different species). In order to express heterologous antigens in a desired attenuated or avirulent pathogen, a gene encoding an antigen capable of providing protection against the pathogen is identified from the deoxyribonucleic acid of a heterologous species. The desired gene is isolated

and then inserted into a plasmid capable of replication and expression in the attenuated or avirulent pathogen. The plasmid is then introduced into the attenuated or avirulent pathogen, and causes expression of the heterologous antigen upon administration to a subject vertebrate.

An example of such expression of an heterologous antigen is the bacterial vaccine *Salmonella*, which expresses a *Streptococcus spa*A protein. See U.S. Patent 4,888,170. This vaccine comprises an avirulent derivative of a pathogenic microbe of the genus *Salmonella*, which in turn expresses a recombinant gene derived from a pathogen of the species *Streptococcus mutans*, thereby producing an antigen capable of inducing an immune response in a vertebrate against the pathogen.

A further example of heterologous expression is *Vibrio cholera* vaccines. A number of live attenuated strains of *Vibrio cholera* have been developed to vaccinate humans against cholera. See Kaper, J.B., et al., New and improved vaccines against cholera in New Generation Vaccines (eds. MM Levin et al.) Marcel Deker, Inc., NY, 1997. Some of these strains over-express heterologous antigens. See Butterton, J.R. and S.B. Calderwood, Attenuated *Vibrio cholera* as a live vector for expression of foreign antigens in New Generation Vaccines (eds. MM Levin et al.) Marcel Deker, Inc., NY, 1997. The immunity induced by the attenuated vaccine strains is the result of inducing antibodies which have either antibacterial and/or antitoxic activities. Some

strains have been attenuated by the deletion of a number of genes encoding toxigenic components, including the A subunit of the cholera toxin encoded by the *ctx*A gene. However, in order for a cholera vaccine strain to be fully protective, it is necessary that the *ctx*B gene encoding the B subunit (to which the A subunit binds) be expressed to allow for the production of antibodies that neutralize the cholera toxin. The *ctx*B gene has been over-expressed in *Vibrio cholera* for the purpose of producing large amounts of the antigen cholera toxin B (CTB). The over-expressed antigen CTB is collected, purified and used as a subunit vaccine which is the extracted CTB antigen. See Lebens M., et al., 1993, Biotechnology (NY) Dec; 11:1574-1578. However, although an over-expressed antigen has itself been used as a vaccine, an attenuated or avirulent pathogen of *Vibrio cholera* which over-expresses the *ctx*B gene, or any other homologous gene, has not been used as a live vaccine.

Another example of heterologous expression is in *Mycobacterium spp*. vaccines, used to prevent tuberculosis in humans. The *Mycobacterium tuberculosis* GroEL protein induces protective immunity when expressed by the *groEL* gene transfected into macrophages (Silva, C.L. and Lowrie, D.B., 1994, Immunology 84:244-248), indicating that GroEL protein is a protective antigen if presented to T cells by this type of antigen presenting cell (APC). Naked DNA vaccines using *Mycobacterium* genes coding for a variety of antigens (hsp70, 85kDa,

65kDa, 36kDa, 6kDa) are also able to induce protective immunity. See Lowrie, D.B. et al., 1997, Vaccine 15:834-838; Tascon, E. et al., 1996, Nat. Med. 2:888-892; and Lozes, E. et al., 1997, Vaccine 15:880-833. It is believed that the naked DNA vaccines work because they transfect APCs (Chattergon, M. et al., 1997, FASEB J. 11:753-763.) which in turn present the antigen appropriately to T cells, thereby inducing a protective cell mediated immunity. *M. bovis* BCG, a live, attenuated strain of *Mycobacterium*, is used to induce protective immunity against *M. tuberculosis* infection in humans. Fine, PM. 1988, Br. Med. Bull. 44:91.

Antigen vaccines developed against Brucellosis provide examples of homologous antigen expression, wherein the antigen is derived from the same species as the attenuated pathogen. Brucellosis is an infectious bacterial disease which can be transmitted to human beings by animals. It is caused by any of a variety of species of pathogenic aerobic bacteria of the genus *Brucella*. In animals, Brucellosis can result in abortion and infertility. In humans, it causes fever, malaise and headaches. This disease has been extensively studied, resulting in the development of numerous vaccines.

It is known that existing vaccine strains of *Brucella*, such as *B. abortus* strains 19 and RB51, and *B. melitensis* strain REV1, can both protect against the *Brucella* species from which they were derived and cross protect against infection by other species, such as *B.*

abortus, *B. melitensis*, *B. ovis*, *B. suis*, *B. canis* and *B. neotomae*. *See* Winter, A.J. et al., 1996, Am. J. Vet. Res., 57:677; P. Nicoletti in Animal Brucellosis, CRC Press (1990), pp. 284-296; J.M. Blasco in Animal Brucellosis, CRC Press (1990), pp. 368-370; and G.C. Alton in Animal Brucellosis, CRC Press (1990), pp. 395-400. New *B. melitensis* strain VTRM1 and *B. suis* strain VTRS1 also cross protect against various *Brucella* species. See Winter, A. J. et al., Am. J. Vet. Res., 57:677.

In the past, one of the most commonly used vaccines to prevent bovine Brucellosis was *B. abortus* strain 19, as described by P. Nicoletti in Animal Brucellosis, CRC Press (1990), pages 284-296. This particular strain of *B. abortus* provided immunity in cattle with a range of protection from 65 to 75% depending upon a number of variables, such as the age of the cattle at vaccination, the dose administered, the route of administration and prevalence of Brucellosis in the vaccinated herd.

B. Abortus strain RB51, a new attenuated live *Brucella* vaccine (marketed as RB-51®), is a stable vaccine approved for use in the United States. See Schurig, G.G. et al, 1991, Vet. Microbiol. 26:359; and Colby, L., 1997, M.Sc. Thesis, Virginia Tech, Blacksburg, Va. Attenuation of strain RB51 is indicated by studies carried out in mice, goats and cattle. See Schurig, G.G., 1991, Vet. Microbiol. 28:171; Palmer R.M. et al., 1997, Am.J. Vet Res. 58:472; Roop, R.M. et al., 1995, Res. Vet. Science, 51:359; and Zambrano, A.J. et

al., 1995, Archivos de Medicina Veterinaria XXVIII, No. extraordinario:119-121. In comparison to the protection provided by strain 19, strain RB51 has been shown in single vaccination protocols to be similarly protective in cattle. See Cheville, N.F. et al., 1993, Amer. J. Vet Research 53:1881; and Cheville, N.F. et al., 1996, Amer. J. Vet Research, 57:1153. Further, oral administration of strain RB51 in mice and cattle has indicated protective immunity. See Stevens, M.G. et al., 1996, Infect. Immun. 64:534. In particular, the mouse model indicates that the protective immunity to Brucellosis induced by strain RB51 is solely T cell mediated because a passive transfer of RB51-induced antibodies does not protect against the disease, whereas adoptive T cell transfer does. See Bagchi, T., 1990, M.Sc. Thesis, Virginia Tech, Blacksburg, Va; Jimenez deBagues, M.P. et al., 1994, Infect. Immun. 62:4990. It is believed that vaccination with RB-51® confers protection by inducing production of interferon gamma able to activate macrophages and specific cytotoxic T cells in the subject which are able to kill *Brucella* infected macrophages.

Although RB-51®, derived from *B. abortus* strain 2308, is the best current vaccine against Brucellosis in animals, it is still not 100% effective. None of the current Brucellosis vaccines are totally effective. Therefore, research continues on promising strains, such as *B. abortus* strain RB51. For example, expression of heterologous antigens by *B. abortus* strain RB51 has been

described by S. Cravero, et al., 1995, Proceedings 4th Intl. Vet. Immunol. Symposium, July, Davis, Ca., Abstract # 276; and S. Cravero et al., 1996, Conference of Research Workers in Animal Diseases, Nov., Chicago, Abstract # 150. Over-expression of a homologous antigen by *Brucella* has been described as a research tool for the purpose of complementing specific deletion mutants for the study of HtrA protein in *B. abortus* (P.H. Elzer, Inf. Immun., 1994, 62:4131), and for the study of physiological functions as discussed by R. Wright at an Oral Presentation of the Brucella Research Conference on Nov. 9, 1997 in Chicago, Ill.

However, over-expression of homologous antigens of *Brucella* or other pathogens, with or without concomitant expression of a heterologous antigen, has not been studied for use in vaccines. Over-expression of homologous antigens previously has been used primarily as a research tool, as described above. An attenuated or avirulent pathogen modified to over-express an homologous antigen has not been used as a live vaccine. However, we have found that a vaccine which is an attenuated or avirulent pathogen which over-expresses one or more homologous antigens, as described herein, will provide greater protection against a pathogenic disease than vaccines of attenuated pathogens which express wild type levels of the same homologous antigens.

Therefore, the invention is directed to a vaccine, a means of producing the vaccine, and its use for

prophylaxis and treatment of a pathogenic disease wherein the vaccine is an attenuated or avirulent pathogen which over-expresses at least one homologous antigen, thereby providing greater protection against and treatment of the disease caused by the unattenuated pathogen in the subject vertebrate.

Summary of the Invention

The invention is directed to a live vaccine which is an attenuated or avirulent pathogen which over-expresses one or more homologous antigens of a pathogen, a method of producing the same, and a method of treating animals, including humans, with the vaccine. This vaccine increases the level of protection against the unattenuated pathogen in comparison to vaccines of attenuated pathogens expressing wild type levels of homologous antigens of the pathogen. In this manner, the over-expressing homologous antigen vaccine will induce a strong cellular mediated immune response and/or a strong humoral antibody response against the unattenuated pathogen in the vaccinated subject.

In particular, it is the purpose of this invention to provide a method of producing a vaccine which is an attenuated or avirulent pathogen over-expressing a homologous antigen, and immunizing an animal, including humans, with the vaccine such that the vaccine induces a strong cell mediated or antibody mediated immune response against a virulent pathogen, thereby providing

complete protection, such as sterile immunity, against a challenge by the virulent pathogen.

It is a further object of the invention to provide a method of producing a vaccine which is an attenuated or avirulent pathogen over-expressing a homologous antigen, and immunizing an animal with the vaccine such that the vaccine causes over-expression of an homologous antigen and expression of a heterologous antigen, both of which provide protection against the virulent pathogen in the vaccinated subject.

It is yet a further object of this invention to provide an over-expressing homologous vaccine, a means for making such a vaccine and a method of using the vaccine for prophylaxis and treatment of Brucellosis in animals, especially bovine animals.

Brief Description of the Drawings

The attached figures are intended to aid in explaining and to more particularly point out the invention described herein. In particular:

Figure 1 is a diagram depicting the derivation of a homologous antigen from a *Brucella* species, and insertion of the antigen into a *Brucella* species vaccine strain;

Figure 2 depicts construction of recombinant plasmids for over-expression of copper/zinc SOD(A) and GroES and GroEL(B) in *B. abortus* strain RB51;

Figure 3 demonstrates the clearance of *B. abortus* strain 2308 from the spleens of mice vaccinated with *B.*

abortus strain RB51 over-expressing copper/zinc SOD or GroES/EL; and

Figure 4 demonstrates the cytotoxic activity by lymphocytes toward Brucella infected cells from mice vaccinated with *B. abortus* strain RB51 over-expressing copper/zinc SOD or GroES/EL.

Detailed Description

The invention is directed to a vaccine for the immunization of vertebrates against disease caused by a pathogen, wherein the vaccine comprises an attenuated or avirulent pathogen that over-expresses one or more homologous antigens encoded by at least one gene from the pathogen, wherein each antigen is capable of inducing a protective immune response against the pathogen.

This over-expressing homologous antigen vaccine is produced by genetic engineering of live, attenuated microbes by a process having the steps of: a) selecting a gene encoding an homologous antigen capable of directly or indirectly stimulating protective immunity against a pathogenic micro-organism (pathogen), and b) inserting said gene into an attenuated or avirulent version of the pathogen such that the homologous antigen is over-expressed. The resultant over-expressing homologous antigen vaccine (OHAV) is more specifically prepared by the following steps:

a) extracting deoxyribonucleic acid from a pathogenic micro-organism;

b) identifying a gene from the deoxyribonucleic acid, wherein said gene encodes at least one antigen capable of stimulating protective immunity against the pathogenic micro-organism;

c) inserting said gene into a plasmid capable of replication and expression in the pathogenic micro-organism; and

d) introducing said plasmid into an attenuated or avirulent version of the pathogenic micro-organism.

The resultant vaccine synthesizes the antigen as a result of transcription and translation of the gene located in at least two sites, i.e., the genome and the plasmid. In particular, it is preferred that the plasmid be a multicopy type, so that it may produce a greater number of the protective antigen than the single genomic copy otherwise generated.

The above method may be used to create over-expressing homologous antigen vaccines for many different diseases. The over-expression of the antigen usually increases both the T cell and antibody immune response, thereby greatly increasing the level of protection in the subject. Because both types of immune response are improved, both intracellular and extracellular pathogens are affected, thereby providing greater protection against the pathogen.

For example, a vaccine against the pathogenic micro-organism *Brucella* may be produced. In particular, the pathogen may be selected from any species of *Brucella*, including *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis*, *B. canis* and *B. neotomae*. The pathogen used to produce the vaccine is preferably selected from a specific strain of *Brucella*, such as *B. abortus* strain

19, *B. abortus* strain RB51, *B. melitensis* strain VTRM1, *B. suis* strain VTRS1 and *B. melitensis* strain REV1.

It is particularly advantageous that the vaccine be prepared with one or more of a Cu/Zn SOD gene, a GroES gene or a GroEL gene of *B. abortus* strain RB51. In particular, it is preferred that the above genes be obtained from a pUC19 genomic library of *B. abortus* strain 2308.

A vaccine produced according to the above specifications is particularly effective for prophylaxis or treatment of diseases such as Brucellosis. For example, an effective vaccine for prophylaxis or treatment of a bovine animal against Brucellosis according to the invention is an attenuated or avirulent derivative of *B. abortus* strain RB51 capable of over-expressing at least one homologous antigen. In particular, it is preferred that the antigen be encoded by one or more of a Cu/Zn SOD gene, a GroES gene or a GroEL gene, preferably selected from a pUC19 genomic library of *B. abortus* strain 2308. It is even more preferable that the attenuated or avirulent derivative also express a heterologous antigen capable of inducing protective immunity against *B. abortus*.

The method of prophylaxis or treatment of a vertebrate suffering from a pathogenic micro-organism is as follows:

a) extract deoxyribonucleic acid from the pathogenic micro-organism;

b) identify at least one gene encoding at least one antigen from the deoxyribonucleic acid, wherein the antigen is capable of stimulating protective immunity against the pathogenic micro-organism;

c) insert the at least one gene into a plasmid capable of replicating and expressing in the pathogenic micro-organism;

d) transform an attenuated or avirulent version of the pathogenic micro-organism with the plasmid to form a vaccine; and

e) administer an effective amount of the vaccine to the vertebrate.

The vaccine used for the method for prophylaxis and treatment may be an original vaccine strain or a modified existing vaccine strain. For example, *B. abortus* strain RB51 can be modified to over-express a homologous antigen, thereby producing a new strain capable of use in a vaccine for the prophylaxis or treatment of Brucellosis, particularly in bovine animals.

In particular, a new *Brucella* vaccine can be prepared by: 1) selecting a gene encoding a protective antigen from a strain of *Brucella*; 2) inserting the gene from the pathogen into a multicopy plasmid capable of replication and expression in *Brucella*; and 3) introducing the plasmid into *Brucella* by means such as transformation. One or more homologous antigens may be over-expressed in this manner. Additionally, one or

more heterologous antigens may be expressed in the vaccine by methods known in the art.

By over-expressing one or more homologous antigens of a given pathogen, greater T cell and/or antibody immune response against that pathogen is stimulated in the vertebrate treated with the vaccine produced from the attenuated or avirulent pathogen, affording greater protection against the unattenuated pathogen. Further protection may be offered by additional expression of one or more heterologous antigens by the attenuated or avirulent pathogen by means known to one of ordinary skill in the art.

The resultant over-expressing homologous antigen vaccine may be administered in a dose effective to promote prophylaxis or treatment of a disease caused by the pathogen in the desired subject vertebrate. As known to one of ordinary skill in the art, dosages should be adjusted for each subject based on factors such as weight, age, and environmental factors. The effective dose may be administered in any effective manner based on the type of animal being treated, its age and condition.

Examples

Example 1:

Two OHAVs were constructed by over-expressing either the Cu/Zn SOD gene or the GroES and GroEL genes in *B. abortus* strain RB51. The genes for Cu/Zn SOD,

GroES and GroEL were initially obtained from a pUC19 genomic library of *B. abortus* strain 2308. As shown in Fig. 2, the inserts containing these genes along with their own promoters were excised from the pBA113 (SOD) and pBA2131 (GroES and GroEL) regions and subcloned into pBBR1MCS, a broad-host range plasmid which has routinely been used in *Brucella* research. The resulting recombinant plasmids were termed as pBBSOD and pBBGroES/EL (Fig. 2). The *B. abortus* strain RB51 was transformed with these plasmids by electroporation. *Brucella* containing the plasmids were selected by plating the transformed bacteria on trypticase soy agar plates containing 30 μg/mL of chloramphenicol. To determine the over-expression of the cloned genes, the antibiotic resistant colonies were individually grown in trypticase soya broth and the bacterial extracts used as antigens in an immunoblot analysis. Strain RB51 containing pBBSOD (RB51SOD) and pBBGroES/EL (RB51GroESL) over-expressed Cu/Zn SOD and GroEL, respectively, as compared to strain RB51 containing pBBR1MCS alone (RB51pBB).

Protection studies in mice:

Groups of 8 mice were vaccinated by inoculating, intraperitoneally, 4 x 10^8 colony forming units (cfu) of either strain RB51SOD, RB51GroESL, RB51pBB or RB51 in 0.5mL of saline. One group of mice was inoculated with 0.5mL of saline as a control. After 6 weeks, 5 mice in each group were challenged intraperitoneally with 2.5 x 10^4 cfu of virulent strain 2308. The remaining three mice in each group were used to characterize the immune responses. Two weeks after challenge with virulent strain 2308, mice were euthanized and the cfu of strain 2308 per spleen were determined. Mice immunized with strain RB51SOD had a significantly lower number of bacteria as compared to those immunized with strain RB51. In mice immunized with strain RB51GroESL, the number of bacteria observable was at the lower limit (<20 cfu/spleen) of the detection method.

Characterization of immune responses

After 6 weeks of vaccination, serum was collected from 3 mice in each group for analysis of the humoral antibody response. These mice were euthanized and the lymphocytes harvested from their spleens were used to study the cell-mediated immune response. As shown in Fig. 3, mice vaccinated with strain RB51 developed antibodies to GroEL but did not develop antibodies to Cu/Zn SOD. In contrast, mice vaccinated with strain RB51SOD developed a strong antibody response to Cu/Zn SOD, and mice vaccinated with strain RB51GroESL

developed a stronger antibody response to GroEL protein (Fig. 3) than that exhibited by strain RB51 vaccinated mice. These results indicate an enhanced antibody response by the OHAV.

The cell mediated immune response caused was characterized by determining the cytotoxic activity of lymphocytes toward *Brucella* infected cells. Specific splenic lymphocyte activity was enhanced in vitro by co-culturing with mitomycin C treated *Brucella* infected macrophages as stimulator cells. A cytotoxicity assay was performed using enhanced lymphocytes as effector cells (E) and *Brucella* infected macrophages as target cells (T). In the assay, E and T cells were mixed in two different ratios, 10:1 and 5:1. The percent specific lysis of target cells was calculated for each E:T ratio using standard methods (Fig 4). Lymphocytes from mice vaccinated with RB51SOD or RB51GroESL showed enhanced cytotoxic activity relative to saline or strain RB51 vaccinated mice. This increased cytotoxic lymphocyte activity (indicated by the increased % specific lysis) directly correlates with the observed enhanced protection of mice against challenge with virulent *B. abortus* strain 2308; the higher the protective level, the higher the specific cytotoxic activity.

Example 2:

An OHAV is constructed by over-expressing the *ctxB* gene in *Vibrio cholera*. The gene is obtained from the deoxyribonucleic acid of the pathogen and inserted into

a plasmid capable of replicating and expressing in the pathogen. The resulting recombinant plasmid is used to transform *Vibrio cholera* by means of electroporation. Plasmids are plated and selected by means known in the art. The resultant over-expressing homologous antigen vaccine strain promotes overproduction of antibodies that neutralize the cholera toxin, thereby providing greater protection for prophylaxis and treatment of cholera in humans.

Example 3:

An OHAV is constructed by over-expressing the *gro*EL gene of *Mycobacterium tuberculosis* in a *Mycobacterium* species. The gene is obtained from the deoxyribonucleic acid of the pathogen and inserted into a plasmid capable of replicating and expressing in the pathogen. The resulting recombinant plasmid is used to transform a *Mycobacterium* species by means of electroporation. Plasmids are plated and selected by means known in the art. The resultant over-expressing homologous antigen vaccine strain promotes overproduction of GroEL proteins, thereby providing greater protection for prophylaxis and treatment of tuberculosis in humans. In particular, over-expression of the *gro*EL gene encoding the GroEL protein in *M. bovis* BCG provides greater protective immunity against tuberculosis because BCG vaccines are known to target antigen protecting cells, such as macrophages, thereby providing a means of

introducing the antigens into the T cells, inducing protective cell mediated immunity.

The above examples are illustrative only. The scope of the invention is not limited to the examples, but is described in the specification and accompanying claims. Those of ordinary skill in the art will recognize methods and materials which could be substituted for those described above, and any such methods and materials are intended to be covered by the above disclosure and following claims.

We claim:

1. A vaccine for the immunization of vertebrates against disease caused by a pathogen, wherein said vaccine comprises an attenuated or avirulent derivative of said pathogen that over-expresses at least one homologous antigens encoded by at least one gene from said pathogen and wherein said at least one antigen is capable of inducing a protective immune response against the pathogen.

2. The vaccine of claim 1, wherein said attenuated or avirulent derivative of said pathogen further expresses one or more heterologous antigens.

3. The vaccine of claim 1, wherein the pathogen is selected from the group consisting of *Brucella*, *Mycobacterium*, and *Vibrio*.

4. A vaccine for prophylaxis or treatment of a vertebrate against Brucellosis, wherein said vaccine comprises an attenuated or avirulent pathogen of *Brucella*, wherein the attenuated or avirulent pathogen over-expresses at least one homologous antigen encoded by at least one gene from said pathogen, and wherein the at least one antigen is capable of inducing a protective immune response in the vertebrate against Brucellosis.

5. The vaccine of claim 4, wherein the pathogen is selected from the group consisting of *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis*.

6. The vaccine of claim 4, wherein *Brucella* is *B. abortus* strain RB51.

7. The vaccine of claim 6, wherein the at least one gene is a Cu/Zn SOD gene.

8. The vaccine of claim 7, wherein the Cu/Zn gene is obtained from a pUC19 genomic library of *B. abortus* strain 2308.

9. The vaccine of claim 6, wherein the at least one gene is one or both of a GroES gene and a GroEL gene.

10. The vaccine of claim 9, wherein the GroES gene and the GroEL gene are obtained from a pUC19 genomic library of *B. abortus* strain 2308.

11. The vaccine of claim 4, wherein the vertebrate is bovine.

12. An attenuated or avirulent version of *B. abortus* strain RB51 that over-expresses at least one homologous antigen capable of stimulating protective immunity against Brucellosis.

13. The attenuated or avirulent version of *B. abortus* strain RB51 of claim 12, wherein the at least one homologous antigen is encoded by at least one gene selected from the group consisting of a Cu/Zn SOD gene, a GroES gene and a GroEL gene.

14. A method for prophylaxis or treatment of a vertebrate at risk of or suffering from a pathogenic micro-organism comprising administering an effective amount of an over-expressing homologous antigen vaccine, wherein said vaccine is an attenuated or avirulent version of said pathogenic micro-organism that over-expresses at least one homologous antigen of the pathogenic micro-organism.

15. The method of claim 14, wherein the vaccine further expresses a heterologous antigen.

16. The method of claim 14, wherein the pathogenic micro-organism is *Mycobacterium* or *Vibrio*.

17. The method of claim 16, wherein the vertebrate is human.

18. A method for prophylaxis or treatment of a vertebrate at risk of or suffering from Brucellosis comprising administering an effective amount of a vaccine, wherein said vaccine is an attenuated or avirulent pathogen of *Brucella* that over-expresses at

least one homologous antigen encoded by at least one gene from said attenuated or avirulent pathogen.

19. The method of claim 18, wherein said attenuated or avirulent pathogen further expresses an heterologous antigen.

20. The method of claim 18, wherein the at least one gene is a Cu/Zn SOD gene in *B. abortus* strain RB51.

21. The method of claim 20, wherein the Cu/Zn gene is obtained from a pUC19 genomic library of *B. abortus* strain 2308.

22. The method of claim 18, wherein the at least one gene is one or both of a GroES gene and a GroEL gene in *B. abortus* strain RB51.

23. The method of claim 22, wherein the GroES gene and the GroEL gene are obtained from a pUC19 genomic library of *B. abortus* strain 2308.

24. A method for prophylaxis or treatment of a vertebrate at risk of or suffering from a pathogenic micro-organism comprising the steps of:

a) extracting deoxyribonucleic acid from the pathogenic micro-organism;

b) identifying at least one gene encoding at least one antigen from the deoxyribonucleic acid, wherein said

at least one antigen is capable of stimulating protective immunity against the pathogenic micro-organism;

c) inserting the at least one gene into a multicopy plasmid capable of replicating and expressing in the pathogenic micro-organism;

d) transforming an attenuated or avirulent version of the pathogenic micro-organism with the plasmid to form a vaccine; and

e) administering an effective amount of said vaccine to the vertebrate.

25. The method of claim 24, wherein said attenuated or avirulent version of the pathogenic micro-organism further expresses one or more heterologous antigens.

26. The method of claim 24, wherein the pathogenic micro-organism is selected from the group consisting of *Brucella*, *Mycobacterium*, and *Vibrio*.

27. The method of claim 26, wherein the pathogenic micro-organism is selected from the group consisting of *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. neotomae* and *B. canis*.

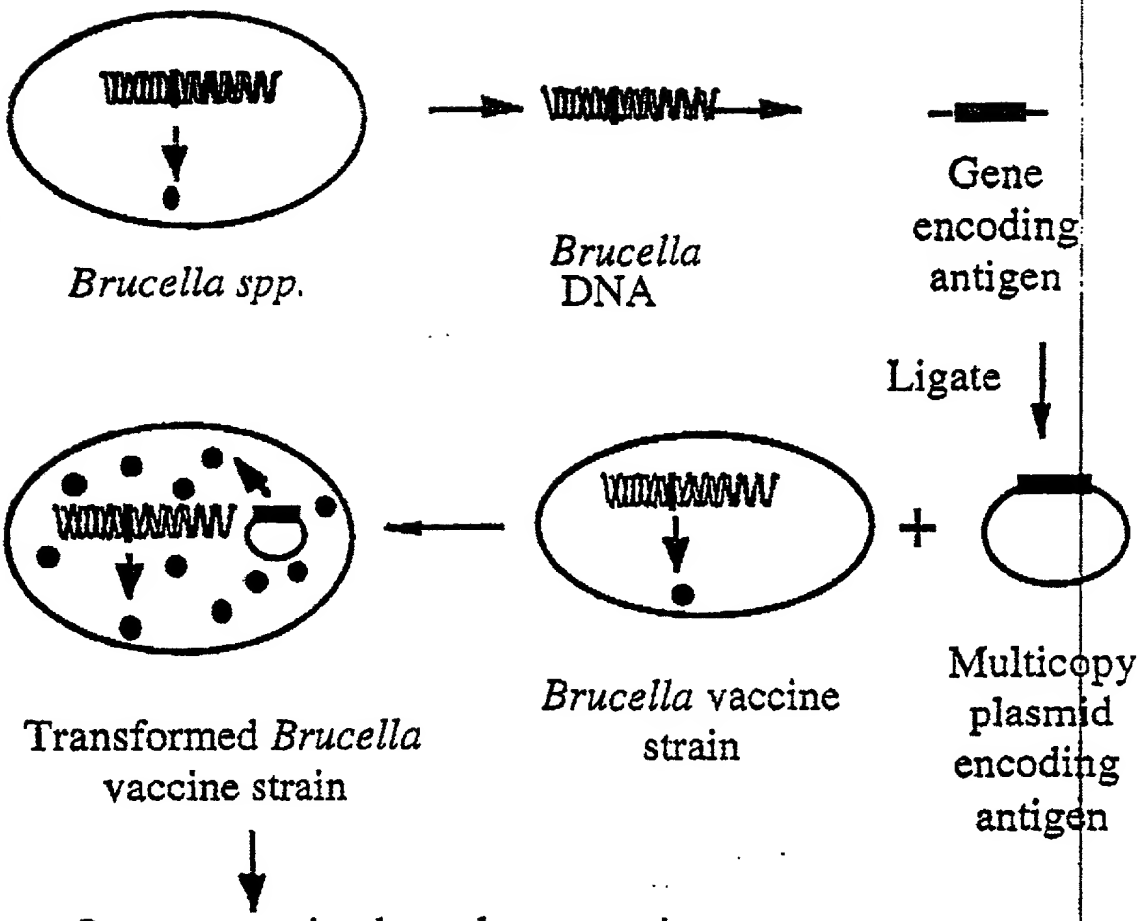
28. The method of claim 27 wherein the pathogenic micro-organism is *B. abortus* strain RB51.

29. The method of claim 28, wherein the at least one gene is a Cu/Zn SOD gene.

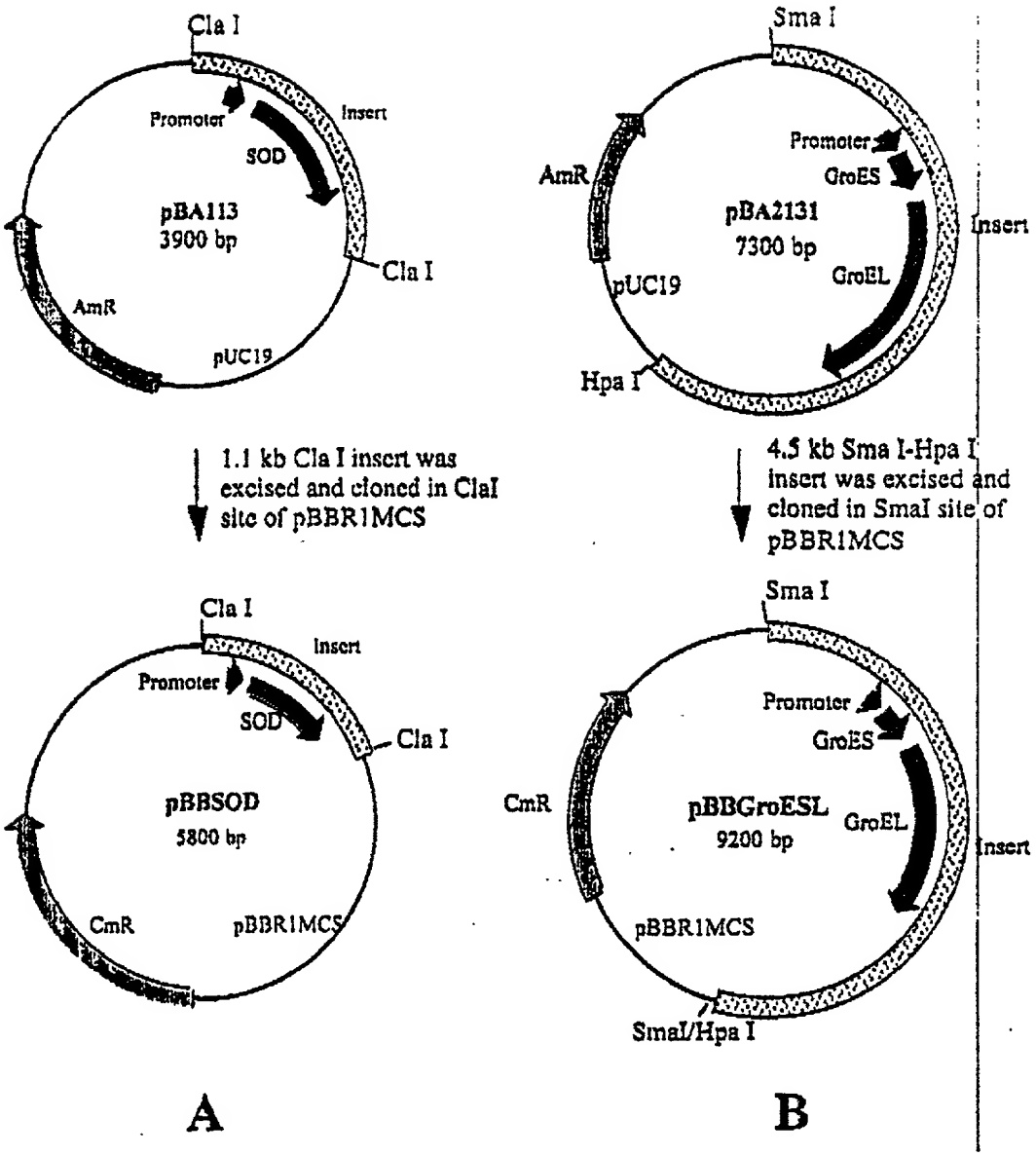
30. The method of claim 28, wherein the at least one gene is one or both of a GroES gene and a GroEL gene.

ABSTRACT

This invention relates to an over-expressing homologous antigen vaccine, a method of producing the same, and use of the vaccine for prophylaxis or treatment of vertebrates at risk of or suffering from disease caused by a pathogenic micro-organism. The vaccine is an attenuated or avirulent pathogenic micro-organism that over-expresses at least one homologous antigen encoded by at least one gene derived from the pathogenic micro-organism, and may also express a heterologous antigen.



Over-expressing homologous antigen vaccine against *Brucella* spp. Note: the gene encoding the homologous antigen is being expressed in the vaccine strain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Sma I
Promoter
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GroEL
Hpa I
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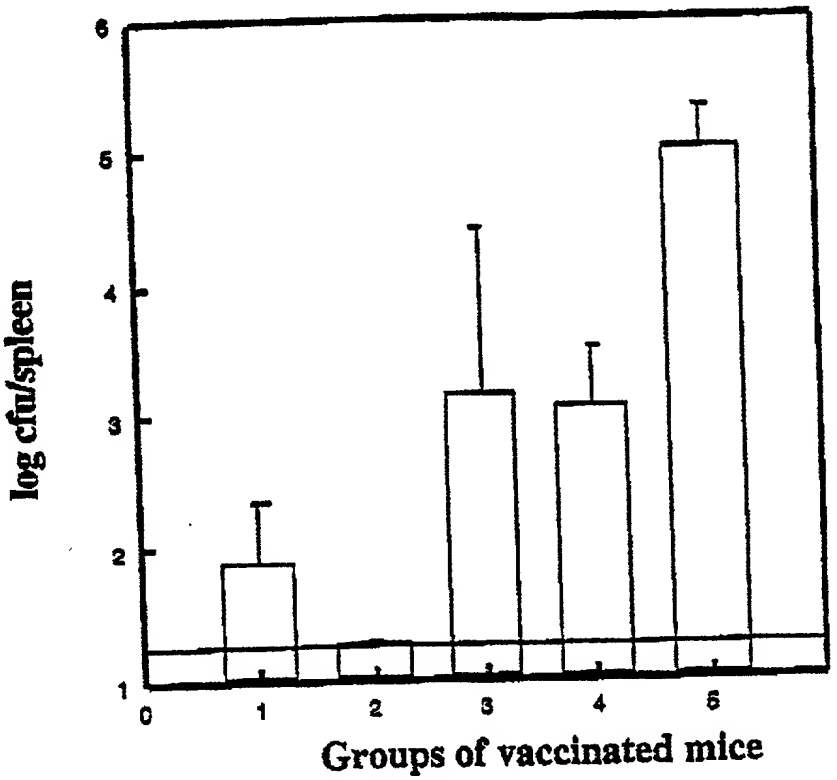
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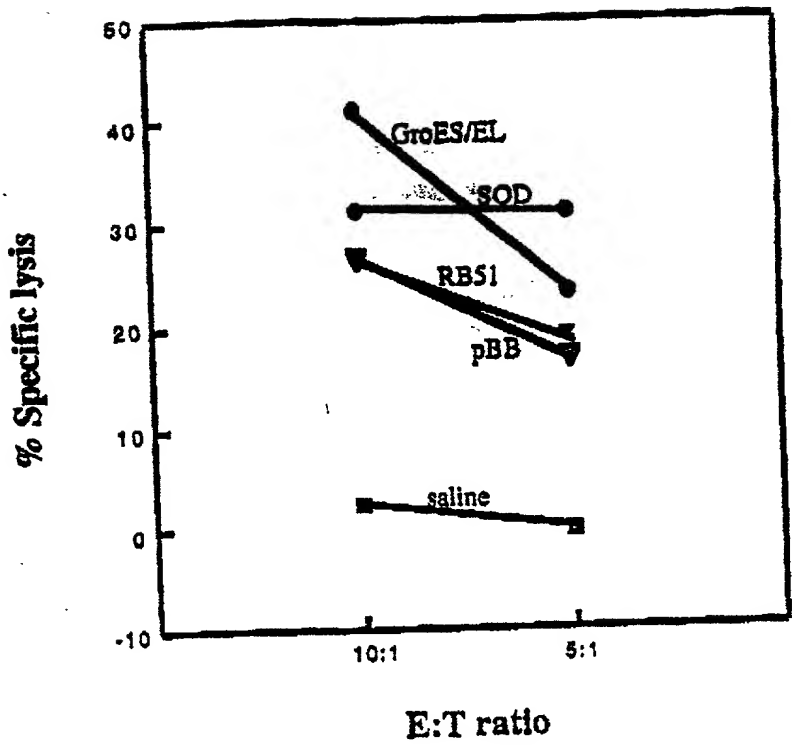
Groups of vaccinated mice
detection limit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% Specific lysis
GroES/EL
SOD
RB51
pBB
saline
10:1
5:1
E:T ratio

COMBINED DECLARATION FOR PAT APPLICATION AND POWER OF ATTORNE (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NUMBER 031786-019

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Number _____

on _____

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/US97/23032

on 05 December 1997

and was amended under PCT Article 19

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I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

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			__Yes __No
			__Yes __No
			__Yes __No
			__Yes __No

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(Application Number) (Filing Date)

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U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S.				
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)		

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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POST OFFICE ADDRESS 2100 Foxhunt Lane NW, Apt. J, Blacksburg, Virginia 24060		
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

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As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Number _____

on _____

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/US97/23032

on 05 December 1997

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

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			__Yes __No
			__Yes __No

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_____ _____
(Application Number) (Filing Date)

COMBINED DECLARATION FOR PAT APPLICATION AND POWER OF ATTORNE. ONTINUED) (Includes Reference to Provisional and PCT International Applications)		ATTORNEY'S DOCKET NO. 031786-019
FULL NAME OF SOLE OR FIRST INVENTOR Stephen M. BOYLE	SIGNATURE	DATE
RESIDENCE 301 Woodbine Drive, Blacksburg, Virginia 24061		CITIZENSHIP United States
POST OFFICE ADDRESS 301 Woodbine Drive, Blacksburg, Virginia 24061		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY Silvio CRAVERO	SIGNATURE	DATE 06/11/98
RESIDENCE Felipe Vallese 773 8B, 1405 Capital Federal, Republica, Argentina		CITIZENSHIP Argentina
POST OFFICE ADDRESS Felipe Vallese 773 8B, 1405 Capital Federal, Republica, Argentina		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY Lynette CORBEIL	SIGNATURE	DATE
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POST OFFICE ADDRESS 1648 Neale Street, San Diego, California 92103		
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY Gerhardt SCHURIG	SIGNATURE	DATE
RESIDENCE 2906 Wakefield Drive, Blacksburg, Virginia 24060		CITIZENSHIP United States
POST OFFICE ADDRESS 2906 Wakefield Drive, Blacksburg, Virginia 24060		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY Nammalwar SRIRNAGANATHAN	SIGNATURE	DATE
RESIDENCE 507 Cedar Orchard Drive, Blacksburg, Virginia 24060		CITIZENSHIP United States
POST OFFICE ADDRESS 507 Cedar Orchard Drive, Blacksburg, Virginia 24060		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY Ramesh VEMULAPALLI	SIGNATURE	DATE
RESIDENCE 2100 Foxhunt Lane NW, Apt. J, Blacksburg, Virginia 24060		CITIZENSHIP United States
POST OFFICE ADDRESS 2100 Foxhunt Lane NW, Apt. J, Blacksburg, Virginia 24060		
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

COMBINED DECLARATION FOR PAT. APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NUMBER 031786-019

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

AN OVER-EXPRESSING HOMOLOGOUS ANTIGEN VACCINE AND A METHOD OF MAKING THE SAME

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Number _____

on _____

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/US97/23032

on 05 December 1997

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
			__Yes __No
			__Yes __No
			__Yes __No
			__Yes __No
			__Yes __No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

_____ _____
(Application Number) (Filing Date)

_____ _____
(Application Number) (Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NO. 031786-019

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.

PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	Robert G. Mukai	28,531	William H. Benz	25,952
Peter H. Smolka	15,913	George A. Hovanec, Jr.	28,223	Peter K. Skiff	31,917
Robert S. Swecker	19,885	James A. LaBarre	28,632	Richard J. McGrath	29,195
Platon N. Mandros	22,124	E. Joseph Gess	28,510	Matthew L. Schneider	32,814
Benton S. Duffett, Jr.	22,030	R. Danny Huntington	27,903	Michael G. Savage	32,596
Joseph R. Magnone	24,239	Eric H. Weisblatt	30,505	Gerald F. Swiss	30,113
Norman H. Stepno	22,716	James W. Peterson	26,057	Michael J. Ure	33,089
Ronald L. Grudziecki	24,970	Teresa Stanek Rea	30,427	Charles F. Wieland III	33,096
Frederick G. Michaud, Jr.	26,003	Robert E. Krebs	25,885	Bruce T. Wieder	33,815
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Samuel C. Miller, III	27,360	Patrick C. Keane	32,858		
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PAT APPLICATION AND POWER OF ATTORNE CONTINUED) (Includes Reference to Provisional and CT International Applications)		ATTORNEY'S DOCKET NO. 031786-019
FULL NAME OF SOLE OR FIRST INVENTOR Stephen M. BOYLE	SIGNATURE Stephen M. Boyle	DATE 6-8-98
RESIDENCE 301 Woodbine Drive, Blacksburg, Virginia 24061		CITIZENSHIP United States
POST OFFICE ADDRESS 301 Woodbine Drive, Blacksburg, Virginia 24061		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY Silvio CRAVERO	SIGNATURE	DATE
RESIDENCE Felipe Vallese 773 8B, 1405 Capital Federal, Republica, Argentina		CITIZENSHIP Argentina
POST OFFICE ADDRESS Felipe Vallese 773 8B, 1405 Capital Federal, Republica, Argentina		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY Lynette CORBEIL	SIGNATURE	DATE
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FULL NAME OF FOURTH JOINT INVENTOR, IF ANY Gerhardt SCHURIG	SIGNATURE	DATE 6/8/98
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FULL NAME OF FIFTH JOINT INVENTOR, IF ANY Nammalwar SRIRNAGANATHAN	SIGNATURE	DATE 6/8/98
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FULL NAME OF SIXTH JOINT INVENTOR, IF ANY Ramesh VEMULAPALLI	SIGNATURE	DATE 6/8/98
RESIDENCE 2100 Foxhunt Lane NW, Apt. J, Blacksburg, Virginia 24060		CITIZENSHIP United States
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FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
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POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

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